# STUDIES ON THE STRUCTURE OF VIOMYCIN

BY

LAWRENCE HAROLD MASON

B. A., Wesleyan University, 1950

R. UNIV. BIBLIOTHEEK LEIDEN

## AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS

1953

# STUDIES ON THE STRUCTURE OF VIOMYCIN

BY

LAWRENCE HAROLD MASON

B. A., Wesleyan University, 1950

## AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS

1953

## ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. H. E. Carter for his friendly interest and valuable guidance throughout this investigation.

The author is indebted to Chas. Pfizer and to Parke, Davis and Company for the supplies of viomycin which made this work possible, and to the Upjohn Company, Eli Lilly and Company, Abbott Laboratories, and the National Science Foundation for their fellowships.

The author wishes to thank Mrs. Peggy Wiegand and Mrs. Harriett Slack for analyses run and Miss Helen Miklas for the infrared spectra.

The author would also like to acknowledge the help and cheerful encouragement of his wife, Joyce.

# STUDIES ON THE STRUCTURE OF VIOMYCIN

## Introduction

Viomycin, a potent, toxic, water-soluble, petide antibiotic, was isolated simultaneously in the laboratories of Chas. Pfizer and Parke, Davis and Company. Currently it has become of interest to this laboratory to continue studies begun by Haskell and co-workers (1) in an effort to add to the knowledge of the metabolic products of the *Streptomyces* genus of microorganisms. Studies were begun simultaneously on a guanido fraction in the acid hydrolysate of viomycin and on the intact molecule.

## Discussion of Results

Viomycin has been assigned a molecular weight of 835 and a molecular formula of $C_{25}H_{44}N_{12}O_{11} \cdot {}^{3}/_{2}H_2SO_4$, based on diaphragm-cell diffusion studies, analytical data, and chemical studies. In acidic and neutral solution viomycin absorbs in the ultraviolet at 268 m$\mu$ with an extinction coefficient of 22,900. In alkaline solution the maximum is shifted to 290m$\mu$ and the extinction to 18,700. Potentiometric titrations and ultraviolet spectra studies revealed the presence of three basic groups with pKa's of 8.2, 10.3, and 12. The pKa of 12 is probably that of the guanido group responsible for the positive Sakaguchi test given by viomycin. No evidence for a free acidic group in the intact molecule was found.

The compounds which have been isolated from the 6 *N* acid hydrolysate of viomycin include L-serine, L-2,3-diaminopropionic acid, $\beta$-lysine, urea, carbon dioxide, ammonia, and a mixture of guanido compounds. In the course of milder acid hydrolyses the Sakaguchi intensity increases to a maximum and then decreases. Concurrent with the increase in the Sakaguchi intensity urea is produced and the ultraviolet absorption is reduced. Hydrolysis with 1 *N* acid produced $\beta$-lysine, serine, urea, small amounts of ammonia and carbon dioxide, and only traces of diaminopropionic acid. Viomycin was hydrolyzed for 5 hours at 95° with 0.1 *N* acid. The urea produced was removed and the urea-free product

was hydrolyzed with 6 *N* acid for 6 hours at 100°. Ammonia and carbon dioxide, which could not have come from urea were produced.

Hydrolysis of viomycin with 0.1 *N* lithium hydroxide at 95° produced ammonia and urea. The Sakaguchi intensity decreased to zero in 15 minutes, though the urea production and the decrease in the ultraviolet absorption took 2 hours.

Acid hydrolysis of 2,4-dinitrophenylviomycin showed that one of the amino groups of β-lysine is free in the intact molecule of viomycin.

Though no evidence for a simple carbonyl group in viomycin could be obtained, viomycin readily reduced four equivalents of neutral permanganate and two equivalents of iodine in bicarbonate solution. Concurrent with the reduction the ultraviolet peak was destroyed. Only slight evidence for a free carboxyl group was obtained on the permanganate oxidation product of viomycin though the pH decreased from 6.0 to 5.5 during the oxidation. Periodic acid oxidations indicated the absence of vicinal hydroxyl and amino groups.

A mixture of basic guanido compounds was isolated from the acid hydrolysate of viomycin with the aid of Amberlite IRA-400 in the hydroxyl phase. Attempts to fractionate the mixture with Solka-floc and with Amberlite XE-64 in the hydrogen phase failed. Some fractionation was achieved with Amberlite XE-67 in the hydroxyl phase. Analyses of the *p*-hydroxyazobenesulfonate and the flavianate of the major XE-67 column fraction indicated a partial empirical formula of $C_6N_4$. The fraction exhibited pKa's of 1.5, 5.7, and 12.4 when titrated potentiometrically. The titration also indicated a molecular weight of 235 for the chloride. A specific rotation of -63° was observed on an aqueous solution of the fraction.

## Summary

1. Viomycin Sulfate has been indicated to have a molecular weight of 835 and a formula of $C_{25}H_{44}N_{12}O_{11} \cdot {}^3/_2H_2SO_4$.

2. Viomycin has three basic groups with pKa's of 8.2, 10.3, and 12, but no free acidic groups.

3. Viomycin contains a group with an absorption maxi-

mum in acidic and neutral solution at 268 mμ with an extinction coefficient of 12,900. The chromophore is destroyed by neutral permanganate, iodine in bicarbonate, strong acid, and dilute alkali.

4. Acid hydrolysis splits viomycin into urea, ammonia, carbon dioxide, L-serine, L-2,3-diaminopropionic acid, β-lysine, and a mixture of guanido compounds.

5. Studies of one of the basic guanido fractions indicated pKa's of 1.5, 5.7, and 12.4, and an optical rotation of -63°.

## Bibliography

1. Haskell, T. H., Fusari, S. A., Frohardt, R. P., and Bartz, Q. R., *J. Am. Chem. Soc.*, **74**, 599 (1952).

## VITA

The author was born in Rochester, Minnesota, on July 16, 1928. He attended elementary schools in that community and was graduated from Rochester High School in June, 1946. From September, 1946, to June, 1950, he attended Wesleyan University, Middletown, Connecticut, with the aid of a Regional Thorndyke Scholarship. In June, 1950, he received the degree of Bachelor of Arts with high honors in scholarship and high distinction in chemistry. In September, 1950, he entered the Graduate College of the University of Illinois, Urbana. For the first two years he held a fellowship sponsored jointly by the Upjohn Company, Eli Lilly and Company, and Abbott Laboratories. The third year he held a National Science Foundation Fellowship. The author was married to Miss Joyce Ann Thurber of Farmer City, Illinois, on June 8, 1952.